Human vascular smooth muscle cell DNA synthesis stimulated by native low density lipoproteins involves redox-sensitive pathways. Inhibition by N-acetylcysteine and green tea constituents

Locher, R

Abstract: High plasma concentrations of LDL are an important cardiovascular risk factor. The mechanisms involved in DNA synthesis induced by native LDL in human vascular smooth muscle cells are unclear. We investigated the potential role of oxidative stress in human vascular smooth muscle cells (VSMC) DNA synthesis using the antioxidant N-acetylcysteine (NAC) and different green tea catechins, i. e. epigallocatechin (EGC), epigallocatechin gallate (EGCg) and green tea polyphenon, a mixture of green tea catechins at physiological concentrations (0.01-1 g/mL). DNA synthesis was determined using radiolabeled thymidine incorporation, oxidative susceptibility of human serum was studied using 2,2'-azobis(2-amidinopropane) (AAPH). Data are expressed as percent of control. LDL (100 g/mL LDL protein) increased DNA synthesis by 165% (from 100±3 to 265±6%, $p<0.05$), an effect that was completely blocked by pretreatment with the antioxidant N-acetylcysteine (98±22%, $p<0.0001$ vs. LDL). Similarly, epigallocatechin and epigallocatechin gallate concentration-dependently inhibited LDL-induced DNA synthesis to 122±4% and 117±4%, respectively ($P<0.001$ vs. LDL). Coincubation of both EGC and EGCg completely prevented LDL-induced DNA synthesis (92±2%, $p<0.001$ vs. LDL). Polyphenon alone reduced to DNA synthesis to 170±6%($p<0.05$ vs. LDL), being less potent than the combination of EGC+EGCg, and also inhibited serum oxidation by 46±8% ($p<0.05$). However, combination of NAC and polyphenon markedly inhibited LDL synthesis below control levels (21±9%, $p<0.0001$ vs. LDL and control). Treatments had no effect on cell viability as determined by LDH release and Tryphan blue tests. These results demonstrate for the first time that LDL-induced DNA synthesis in human vascular smooth muscle cells involves redox-sensitive pathways that can be inhibited by physiological concentrations of green tea catechins and/or non-specific antioxidants. Antioxidant therapy may therefore be beneficial in inhibiting the growth-promoting effects of native LDL in human vascular smooth muscle cells

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POSSIBLE MECHANISMS OF ANTIHYPERTENSIVE EFFECT OF GARLIC: EVIDENCE FROM MAMMALIAN EXPERIMENTAL MODELS

John A.O. Ojewole, Clement O. Adewunmi. [1]Department of Pharmacology, Faculty of Health Sciences, University of Durban-Westville, Durban, South Africa, [2]DRPU, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

Garlic [*Allium sativum* Linn.(family: Alliaceae) bulb] is perhaps the most widely quoted herb with medicinal values in the medical literature. From alternative and ayurvedic medicine, garlic is known to decrease arterial blood pressure, reduce blood fat and cholesterol, inhibit platelet aggregation and to possess antibacterial and antifungal properties. It has been claimed that the main bioactive compound in garlic extracts is the sulphur-containing allicin, although several other compounds have been isolated from the herb. To date, the precise mechanism of the hypotensive effect of garlic in man and laboratory mammals still remains speculative. The present study was undertaken to shed light on the possible mechanisms of antihypertensive action of garlic, using mammalian experimental models. Garlic methanolic extract (GME, 50-800 mg/ml) significantly ($P< 0.05$ - 0.001) reduced or abolished in a concentration-dependent fashion, the positive inotropic and chronotropic responses of guinea-pig isolated atrial muscle preparations induced by noradrenaline or isoprenaline (10-100 μM), and calcium (Ca^{2+}, 5-40 mM). At the same concentration levels, the plant extract significantly ($P< 0.05$-0.001) inhibited or abolished, in a concentration-related manner, the spontaneous, myogenic contractions of rat isolated portal vein, and also significantly ($P< 0.05$-0.001) reduced or abolished contractions of the rat isolated aortic rings and portal veins provoked by bath-applied noradrenaline (10-100 μM). Moreover, garlic methanolic extract (GME, 50-800 mg/kg p. o.) significantly ($P< 0.05$-0.001) and dose-dependently reduced systemic arterial blood pressures and heart rates of conscious renovascular hypertensive rats. The cardiodepressant effects of garlic *in vitro* were not modified by exogenous adminstration of atropine (10-80 μM) to the bath fluid, but partially attenuated by bath-applied $\pm$-propanolol (10-80 μM). Similarly, the inhibitory effects of the herb on adrenergic agonist-induced contractions of the vascular smooth muscles examined were only partially antagonised by $\pm$-propranolol (10-80 μM). The hypotensive effect of the herb *in vivo* in conscious renovascular hypertensive rats were not affected by pre-treatment of the animals with atropine or chlorpheniramine (10-40 mg/kg p. o.). The results of this study suggest that the cardiodepressant effect of garlic may be due in part to β_1-adrenoceptor blockade, and partly as a consequence of the non-specific spasmolytic property of the plant extract. The inhibitory effects of the plant extract on the isolated vascular smooth muscles examined is likely to be due to its direct vasodilatation activity. It is unlikely, however, that the herb produces hypotension through cholinergic or histaminergic mechanisms since its hypotensive effects in the conscious rats used were not modified by pre-treatment of the animals with atropine or chlorpheniramine. However, the ability of the plant extract to relax blood vessels directly, thereby causing a reduction in vascular resistance and a subsequent fall in total peripheral resistance, could contribute substantially to the antihypertensive action of the herb. Furthermore, since nitric oxide (NO) has been closely linked to hypotension, it is not unlikely that the plant extract produces hypotension in the experimental mammals used, partly also through its ability to activate nitric oxide synthase (NOS), the enzyme which produces nitric oxide (NO). Nevertheless, the experimental evidence obtained in this study indicates that garlic produces bradycardia, vasodilatation and hypotension, and thus suggests that the herb may be used as a natural supplementary remedy in certain cases of hypertension and cardiac disorders.

Key Words: Alternative Medicine, Garlic, Antihypertensive Effect

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HUMAN VASCULAR SMOOTH MUSCLE CELL DNA SYNTHESIS STIMULATED BY NATIVE LOW DENSITY LIPOPROTEINS INVOLVES REDOX-SENSITIVE PATHWAYS. INHIBITION BY N-ACETYLCYSTEINE AND GREEN TEA CONSTITUENTS

Rudolf Locher, Jian Zhang, Paolo M. Suter, Wilhelm Vetter, Matthias Barton. [1]Department of Medicine, University Hospital Zurich, Zurich, Switzerland

High plasma concentrations of LDL are an important cardiovascular risk factor. The mechanisms involved in DNA synthesis induced by native LDL in human vascular smooth muscle cells are unclear. We investigated the potential role of oxidative stress in human vascular smooth muscle cells (VSMC) DNA synthesis using the antioxidant N-acetylcysteine (NAC) and different green tea catechins, i. e. epigallocatechin (EGC), epigallocatechin gallate (EGCg) and green tea polyphenon, a mixture of green tea catechins at physiological concentrations (0.01-1 g/mL). DNA synthesis was determined using radiolabeled thymidine incorporation, oxidative susceptibility of human serum was studied using 2,2'-azobis(2-amidinopropane) (AAPH). Data are expressed as percent of control. LDL (100 μg/mL LDL protein) increased DNA synthesis by 165% (from 100 ± 3 to $265\pm6\%$, $p<0.05$), an effect that was completely blocked by pretreatment with the antioxidant N-acetylcysteine ($98\pm22\%$, $p<0.0001$ vs. LDL). Similarly, epigallocatechin and epigallocatechin gallate concentration-dependently inhibited LDL-induced DNA synthesis to $122\pm4\%$ and $117\pm4\%$, respectively ($P<0.001$ vs. LDL). Coincubation of both EGC and EGCg completely prevented LDL-induced DNA synthesis ($92\pm2\%$, $p<0.001$ vs. LDL). Polyphenon alone reduced to DNA synthesis to $170\pm6\%$($p<0.05$ vs. LDL), being less potent than the combination of EGC+EGCg, and also inhibited serum oxidation by $46\pm8\%$ ($p<0.05$). However, combination of NAC and polyphenon markedly inhibited LDL synthesis below control levels ($21\pm9\%$, $p<0.0001$ vs. LDL and control). Treatments had no effect on cell viability as determined by LDH release and Tryphan blue tests.

These results demonstrate for the first time that LDL-induced DNA synthesis in human vascular smooth muscle cells involves redox-sensitive pathways that can be inhibited by physiological concentrations of green tea catechins and/or non-specific antioxidants. Antioxidant therapy may therefore be beneficial in inhibiting the growth-promoting effects of native LDL in human vascular smooth muscle cells.

Key Words: green tea, atherosclerosis, oxidative stress

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DIFFERENTIAL EFFECTS OF ACUPUNCTURE ON CLINIC AND 24 HOUR AMBULATORY BLOOD PRESSURE: THE NEED FOR HIGH STANDARDS IN ALTERNATIVE MEDICINE RESEARCH

Richard C. Robinson, Zhongyun Wang, Lei Zhang, Wanpen Vongpatanasin, Cori Mooring, Maryem Kara, Robert J. Gatchel, Ronald G. Victor, Norman M. Kaplan. [1]UT Southwestern Medical Center, Dallas, TX, United States

Traditional Chinese medicine and a large Russian literature advocates the use of acupuncture not only to induce analgesia but also to treat primary hypertension. Before advocating acupuncture as an effective complementary/alternative medicine strategy for treating hypertension or other chronic cardiovascular diseases, it is necessary to demonstrate that the beneficial effects of acupuncture are scientifically robust, long-lasting, and explicable in terms of modern scientific mechanisms. In spontaneously hypertensive rats, acupuncture-like electrical stimulation of thinly myelinated somatic afferents activates central endorphin pathways that elicit long-lasting sympathetically-mediated decreases in blood pressure (BP), whereas, in normotensive rats,

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